Chronic C75 treatment of diet-induced obese mice increases fat oxidation and reduces food intake to reduce adipose mass

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Departments of 1Pathology, 2Neuroscience, 3Psychiatry, 4Neurology, 5Oncology, and 6Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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Thupari, Jagan N., Eun-Kyoung Kim, Timothy H. Moran, Gabriele V. Ronnett, and Francis P. Kuhajda. Chronic C75 treatment of diet-induced obese mice increases fat oxidation and reduces food intake to reduce adipose mass. Am J Physiol Endocrinol Metab 287: E97–E104, 2004.—Obesity and its attendant disorders, such as type 2 diabetes, are global health problems. We previously reported that C75, an inhibitor of fatty acid synthase (FAS) and stimulator of carnitine palmitoyltransferase I (CPT I), caused anorexia and profound weight loss in lean and genetically obese mice. To approximate human obesity, we utilized a chronic C75 treatment model for diet-induced obese (DIO) mice. Chronic C75 treatment decreased food consumption and increased energy expenditure due to increased fatty acid oxidation in both DIO and lean mice. There was a substantial loss of adipose tissue and resolution of hepatic steatosis in C75-treated DIO mice. Analysis of changes in the expression of hypothalamic neuropeptides demonstrated that the reduced food consumption in C75-treated DIO mice was accompanied by an increase in cocaine and amphetamine-related transcript expression but not by changes in neuropeptide Y such as seen with acute C75 treatment of lean mice. Inhibition of FAS and stimulation of CPT I provide a means to achieve stable, sustained weight loss in DIO mice.

fatty acid synthase; carnitine palmitoyltransferase I; neuropeptide Y

AN ACCEPTABLE PHARMACOLOGICAL APPROACH for weight control has become an overriding priority, because the prevalence of obesity and its associated diseases is rising in both industrial and developing countries worldwide (10, 15). Recently, fatty acid synthase (FAS), the key fatty acid synthetic enzyme (23), emerged as a potential target for the pharmacological manipulation of appetite and weight control (11, 14, 19). Initially conceived by us as a FAS inhibitor (7), C75 has been shown by our group and others to reduce food consumption in lean and leptin-deficient (ob/ob) obese mice in acute treatment experiments (11, 19).

Recently, we reported that, in addition to reducing food consumption, C75 increases fatty acid oxidation in diet-induced obese (DIO) mice through the stimulation of carnitine palmitoyltransferase I (CPT I), the pace-setting enzyme of mitochondrial fatty acid oxidation (12). Thus, in addition to its inhibitory effect on FAS, C75 can act via CPT I to increase fatty acid oxidation in the setting of decreased food intake, during which energy expenditure usually falls (21). This newly identified function of C75, in conjunction with its role as an inhibitor of FAS, likely accounts for the additional weight loss in C75-treated animals over that in pair-fed controls.

Any strategy for obesity therapy will likely require chronic treatment with a relatively selective loss of adipose tissue. To that end, the goal of this study was to develop a 1-mo chronic C75 treatment model suitable for both DIO and lean mice and to examine its effect on weight loss, food consumption, energy production, adiposity, and hypothalamic neuropeptide expression. Our results show that chronic C75 treatment leads to a greater loss of adipose tissue in DIO mice compared with lean mice, as evidenced by increased weight loss, decreased food intake, increased fatty acid oxidation, and reduced adipose mass and hepatic steatosis. Of significance is that C75 has qualitatively different effects on neuropeptide expression in DIO vs. lean mice. Thus pharmacological manipulation of FAS and CPT I remains efficacious for appetite and weight regulation despite the differences in hypothalamic peptide expression between DIO and lean mice.

MATERIALS AND METHODS

DIO and lean mice. All animal experimentation was done in accordance with guidelines on animal care and use as established by the Johns Hopkins University School of Medicine Institutional Animal Care and Use Committee. Twelve-week-old DIO C57BL/6J male mice were obtained from The Jackson Laboratory (Bar Harbor, ME). DIO mice, postweaning, were fed throughout the experiment a synthetic diet (D12492; Research Diets, New Brunswick, NJ) comprised of 60% calories from fat, 20% from carbohydrate, and 20% from protein (5.2 kcal/g). Twelve-week-old C57BL/6J male mice, obtained from The Jackson Laboratory and fed a diet of rodent chow comprised of 13% calories from fat, 58% from carbohydrate, and 29% from protein (4.1 kcal/g), were used for lean animal studies (Prolab RMH 2500; PMI Nutrition International, Brentwood, MO). Mice were maintained in a 12:12-h light-dark cycle at 25°C for 1 wk before treatment for acclimatization. All manipulations of the mice, including weighing, feeding, and treatment, occurred at 9:00 AM, ~3 h past lights-on time.

One-month chronic treatment model. Ten DIO or lean mice were used for each treatment group (control, pair-fed, and C75 treatment). C75 was dissolved in RPMI 1640 (GIBCO-BRL, Life Technologies, Rockville, MD) and injected intraperitoneally every other day at doses indicated. Control and pair-fed mice received intraperitoneal injections of vehicle only. Animals pair-fed to amounts consumed by the C75-treated animals received all their food in a single meal at 9:00 AM, ~3 h past lights-on time. Animal weights and food consumption were measured daily. After completion of the treatment course, animals were euthanized with CO2 at 4 h after the final dose of C75.

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Acute treatment model. Six DIO or lean mice were used for each group. For acute treatment, animals received C75 or vehicle control (20 mg/kg ip), and a third group was food restricted for 23 h. Food restriction was utilized in place of pair-feeding in the acute studies, as pair-feeding produced marked variation in neuropeptide analysis because of the random timing of feeding by the animals. Animals were euthanized 23 h after C75 treatment.

Whole animal calorimetry. $O_2$ consumption ($V_{O_2}$) and CO$_2$ production ($V_{CO_2}$) were measured in 12 mice at a time (4 for each treatment group: C75 treatment, pair fed, or control) with indirect calorimetry (Oxymax Equal Flow System, Columbus Instruments, Columbus, OH). Measurements of respiratory exchange ratio (RER) and $V_{O_2}$ (in ml·kg$^{-1}$·h$^{-1}$) were performed and recorded every 45 min. RER was calculated by Oxymax software, version 5.9, and is defined as the ratio of $V_{CO_2}$ (in ml·kg$^{-1}$·h$^{-1}$) to $V_{O_2}$ (in ml·kg$^{-1}$·h$^{-1}$). During the first 24 h, baseline readings for all animals were recorded. C75-treated animals received 10 mg/kg ip at the beginning of day 2, along with vehicle treatment of controls. Pair-fed animals were food restricted 24 h later, at the beginning of day 3, with an accompanied vehicle treatment.

RNA preparation and Northern blot analysis. Hypothalamic slices of DIO or lean mice from 2- or 4-wk treatments, as described above, were dissected by using the optic chiasm rostrally and the mammillary body caudally as landmarks. Tissues were harvested to a depth of 2 mm and immediately frozen in liquid nitrogen. Total RNA was purified using TRIzol reagent (GIBCO-BRL, Life Technologies, Rockville, MD), and Northern blot analysis was performed as previously described, with 15 μg of total RNA per lane (6). RNA was separated on a 1% agarose MOPS-formaldehyde gel and transferred to Hybond N+ membrane (Amersham Pharmacia Biotech, Piscataway, NJ). RNA was prehybridized for 1 h at 42°C in UltraHyb solution (Ambion, Austin, TX) and then hybridized with random primed $^3$P-labeled DNA probes (10$^6$ cpm/ml hybridization buffer, Amersham-Pharmacia Labeling Kit, Piscataway, NJ) from the cloned plasmids of mouse agouti-related protein (AGRP, U-89486) (3), human neuropeptide Y (NPY, XM-004941) (13), rat cocaine and amphetamine-related transcript (CART, U-10071) (4), and mouse proopiomelanocortin (POMC, AH-005319) (22). The data were normalized to mouse GAPDH, which was hybridized on the same blots. Signals were quantified using a Storm image analyzer with Imagequant software (Molecular Dynamics, Sunnyvale, CA).

Body composition analysis of DIO and lean mice. Five DIO and 5 lean mice from each of the 1-mo treatment groups were frozen in liquid nitrogen after euthanization and shipped to Mr. D. Trokhman at Proctor and Gamble Pharmaceuticals (Cincinnati, OH) for measurement of body fat with magnetic resonance spectroscopy (MRS). All spectra were acquired with a Bruker Biospec Avance spectrometer operating at 200.4 MHz (4.7 Tesla) and ParaVision 2.1.1 software. Each spectrum was processed, and the peak areas were measured using the XwinNmr 2.6 software. Percentages of fat and fat-free mass were determined from each NMR spectrum by the equations of Myskowski et al. (16).

Measurement of hepatic triglyceride. At the conclusion of the 1-mo treatment of DIO and lean mice, livers from five animals from each treatment group were removed and snap-frozen in liquid nitrogen. Total lipids were extracted from ~400 mg (wt wt) of liver tissue (5) and measured using the Wako L-type TG H Kit (Wako Chemicals, Richmond, VA) per the manufacturer’s protocol with minor modifications (2).

Statistical analysis. All data are presented as means ± SE from multiple determinations. Data were analyzed by linear regression, two-tailed unpaired t-tests, or one-way ANOVA, where applicable, with Prism 4.0 (GraphPad Software).

Miscellaneous methods and chemicals. Mouse tissues for histology were fixed in neutral-buffered formalin, paraffin-embedded, and sectioned at 4 μm for hematoxylin and eosin staining. C75 was obtained from FASgen (Baltimore, MD).

RESULTS

Chronic C75 treatment of DIO and lean mice leads to sustained weight loss compared with pair-fed mice. We and others (11, 19) have shown that C75 causes significant weight loss in acutely treated lean and genetically obese (ob/ob) mice. To more closely approximate a human model of obesity for the purpose of investigating treatment strategies, we utilized an accepted DIO mouse model (21) and used doses of C75 that reduced, but did not eliminate, food intake. The goal of this study was to achieve a sustained, stable weight loss with C75 treatment.

The C75-treated DIO mice lost significant body mass (−10.3 ± 2.2%) compared with a slight gain in body mass for the pair-fed animals (+1.4 ± 3.8%), whereas controls gained significantly (+35.1 ± 3.3%; Fig. 1A, Table 1). Thus, similar to what has been shown in short-term C75-treated DIO animals (21), chronic C75 treatment resulted in the loss of more body mass than pair-fed control treatment. In addition, the C75-treated animals consumed significantly less food on average per day (9.8 ± 0.6 kcal·mouse$^{-1}$·day$^{-1}$) compared with the vehicle controls (15.01 ± 0.3 kcal·mouse$^{-1}$·day$^{-1}$), demonstrating the anorexic effect of C75 (Fig. 1B, Table 1). Interestingly, the loss of body mass in the C75-treated animals stabilized at ~10% at this dose of C75 during the last 14 days of the experiment. Similarly, food consumption also plateaued during the last 14 days, reversing the trend of reduced food consumption seen during the first 16 days.

Lean mice also incurred a significant loss of body mass with C75 treatment (−13.8 ± 2.7%) compared with that seen in the pair-fed mice (−3.8 ± 2.5%), whereas controls showed an increase (+9.1 ± 1.7%; Fig. 1C, Table 1). Food consumption was also reduced significantly in the C75-treated mice (10.5 ± 0.5 kcal·mouse$^{-1}$·day$^{-1}$) compared with controls (16.5 ± 0.4 kcal·mouse$^{-1}$·day$^{-1}$; Fig. 1D, Table 1). Most of the weight loss in the lean mice occurred during the first 14 days of treatment. The reduction in food consumption occurred promptly within the first days of treatment and remained reduced, with a trend toward increased food consumption during the last 10 days of treatment.

Importantly, chronically C75-treated mice showed no evidence of central or peripheral neurotoxicity. Stool and urine production was in keeping with food and water consumption; no diarrhea or excessive urination was noted. Thus the nonspecific neuronal stimulation seen in a recent in vitro study; has not been corroborated in vivo (20).

C75 causes increased fatty acid oxidation in DIO and lean mice. We have previously demonstrated that C75 increases fatty acid oxidation in vitro and in short-term (24-h) in vivo studies with DIO mice (21). In this experiment, we monitored RER and $V_{O_2}$ in both lean and DIO mice after the same dose of C75 that was used in the 1-mo studies (10 mg/kg). Figure 2 graphically depicts the averaged raw data from the calorimeter for the four animals in each treatment group. In C75-treated DIO mice, $V_{O_2}$ was increased, compared with controls, but did not reach statistical significance (Fig. 2A). RER was significantly reduced in the C75-treated animals during day 2 compared with controls, indicating increased fatty acid oxidation ($P = 0.0031$; Fig. 2C). More important, however, was the contrast between C75-treated and pair-fed mice. In C75-treated DIO mice, $V_{O_2}$ was significantly increased over
Fig. 1. Chronic C75 treatment of diet-induced obese (DIO) and lean mice induces weight loss and reduced food intake. A: daily weights of C75-treated (red line), pair-fed (blue line), and vehicle control (black line) DIO mice (n = 10 per group). C75-treated mice lost 10.3% of body mass compared with weights on day 0 and differed by 45.4% from controls (P < 0.0001, unpaired t-test) and by 8.9% from pair-fed mice (P = 0.013, unpaired t-test) by day 30. B: daily food consumption (kcal·mouse⁻¹·day⁻¹) in C75-treated DIO (red line) and vehicle control (black line) mice. C75 treatment reduced average daily food consumption (9.8 ± 0.6) compared with vehicle controls (15.0 ± 0.3; P < 0.0001, unpaired t-test). C: daily weights of C75-treated (red line), pair-fed (blue line), and vehicle control (black line) lean mice (n = 8 per group). C75-treated mice lost 13.8% of body mass compared with day 0 and differed by 22.9% from controls (P < 0.0001, unpaired t-test) and by 10% from pair-fed mice (P < 0.0001, unpaired t-test) by day 30. D: daily food consumption (kcal·mouse⁻¹·day⁻¹) in C75-treated lean (red line) and vehicle control mice (black line). C75 treatment reduced average daily food consumption (10.5 ± 0.4) compared with vehicle controls (16.5 ± 0.4; P < 0.0001, unpaired t-test). Arrowheads, C75 treatment days. Mice received 7.5 mg/kg C75 on starred days (P = 0.0001 vs. control). *P < 0.0092, the first day of food restriction for the pair-fed animals (Fig. 2B). RER was similar in both groups (~0.7) over this time period, indicating oxidation of fatty acid (Fig. 2D). Taken together, these data demonstrate that C75-treated animals significantly increased energy expenditure as fatty acid oxidation in the setting of reduced caloric consumption. The increased fatty acid oxidation in C75-treated animals likely accounts for their increased weight loss compared with pair-fed animals. Importantly, the physical activity of the animals was not increased after C75 treatment, which could have accounted for the increased energy expenditure.

Compared with the DIO mice, the lean mice showed a more pronounced diurnal variation in both V̇O₂ and RER, regardless of the treatment group (Fig. 2, E–H), and the baseline RER was higher in the lean animals (~0.85), reflecting their low-fat, high-carbohydrate diet. There were no statistically significant alterations in V̇O₂ among any of the treatment groups (Fig. 2, E and F). In contrast, RER dropped precipitously and significantly during day 1 of C75 treatment compared with the control mice, indicating increased fatty acid oxidation (P < 0.0001; Fig. 2G). When lean C75-treated and pair-fed mice were compared, the magnitude of the RER reduction was the same in both C75 (day 1) and pair-fed mice (day 2) (~0.85 to 0.70), but it persisted almost twice as long in the C75-treated animals, leading to a significant statistical difference (P = 0.042; Fig. 2H). Interestingly, the difference in weight loss between the pair-fed and C75-treated animals was not statistically different between DIO (11.7%) and lean (10.8%) mice.

C75 reduced fat mass in DIO but not lean mice. Any strategy for obesity therapy should selectively target adipose tissue, sparing as much lean mass as possible. Using MRS of

<table>
<thead>
<tr>
<th>Table 1. Body weights of DIO and lean mice</th>
<th>Average Beginning and Final Weights, g</th>
<th>Weight Change, g (%)</th>
<th>Food Consumption, kcal·mouse⁻¹·day⁻¹</th>
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<tr>
<td>DIO</td>
<td></td>
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<tr>
<td>Control</td>
<td>33.6 ± 1.1–45.4 ± 1.5</td>
<td>+11.8 ± 1.5</td>
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<td>C75</td>
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<td>−3.7 ± 0.7</td>
<td>9.8 ± 0.6*</td>
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<tr>
<td>Pair fed</td>
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<td>(1.4 ± 3.8)</td>
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<tr>
<td>Lean</td>
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<td>16.5 ± 0.4</td>
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<tr>
<td>C75</td>
<td>32.5 ± 0.3–28.1 ± 0.7</td>
<td>−4.5 ± 0.1</td>
<td>10.5 ± 0.5*</td>
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<tr>
<td>Pair fed</td>
<td>30.6 ± 0.5–29.4 ± 0.7</td>
<td>−1.2 ± 0.8</td>
<td>(−3.8 ± 2.5)</td>
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Values are means ± SE. DIO, diet-induced obese. *P < 0.0001 vs. control.
DIO and lean mice treated with C75, we sought to determine whether the loss of body mass came from fat or lean tissues. Five mice from each treatment group from the 1-mo experiment were used for the analysis. Fat accounted for 16.7 g or 32.7% of body mass of control DIO mice (Fig. 3A). C75 treatment reduced the average fat mass to 4.3 g (12.7% of total body mass), whereas the pair-fed mice had a fat mass of 7.9 g (18.7% of total body mass; Fig. 3A). Thus most of the reduction of body mass in C75-treated mice represented a reduction in fat mass, not lean mass. Lean control mice were nearly 10-fold leaner than their DIO counterparts. Fat comprised only 1.1 g (3.8%) of the body mass of lean controls (Fig. 3B). There were no significant differences in fat mass between C75 and pair-fed mice. Thus the reduction in body mass in C75-treated lean mice is due to a loss of predominantly lean mass.

C75 reverses hepatic steatosis. Obese humans often develop hepatic steatosis, which may lead to steatohepatitis, and eventually to cirrhosis and liver failure (17). DIO mice can develop a hepatic steatosis similar to that of obese human subjects. Histological examination of the livers of vehicle control DIO mice from the 1-mo C75 treatment study (Fig. 4A) showed intracytoplasmic large and small droplet fat accumulation (arrows), similar to the steatosis seen in humans. Steatosis was

Fig. 2. C75 increases fatty acid oxidation in DIO and lean mice. Average oxygen consumption ($\dot{V}_O_2$) and respiratory exchange ratio (RER) of the 4 mice per each treatment group for DIO and lean mice. Control, C75, and pair-fed mice were measured simultaneously. A: energy consumption as measured by $\dot{V}_O_2$ was similar in both C75 (red line) and control (black line) DIO mice. B: compared with pair-fed mice (blue line), C75 treatment (red line) increased $\dot{V}_O_2$ consumption during day 2 ($P = 0.009$, unpaired t-test), indicating increased energy production. C: RER was significantly increased in control mice (black line) compared with the C75-treated group (red line) during day 2 ($P = 0.007$, unpaired t-test), indicating increased fatty acid oxidation in C75-treated mice. D: RER was similar for C75-treated (red line) and pair-fed mice (blue line), particularly during day 2, when pair-fed mice were food restricted. E and F: energy consumption as measured by $\dot{V}_O_2$ was similar in C75 (red line), control (black line), and pair-fed (blue line) lean mice. G: RER was significantly increased in control mice (black line) compared with the C75-treated group (red line) during day 1 ($P < 0.0001$, unpaired t-test). H: in C75-treated mice (red line), RER was maximally reduced on day 1 and for a significantly longer duration than in pair-fed mice, which were food restricted 24 h later (day 2) ($P = 0.042$, unpaired t-test). These data indicate a statistically significant increase in fatty acid oxidation in the C75-treated mice over pair-fed controls. Values are means ± SE. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Fig. 3. C75 reduces fat mass in DIO but not lean mice, shown by magnetic resonance spectrographic (MRS) analysis of DIO and lean mice after 1 mo of C75 treatment to determine fat mass. A: control DIO mice had a significantly greater fat mass (32.7% of total body mass) than C75-treated mice (12.7% of total body mass; $P = 0.0042$; $n = 5$ animals per group). Although the pair-fed animals also had more fat than C75-treated animals, the difference was not statistically significant. B: control lean mice had significantly less fat mass than DIO controls. There were, however, no statistically significant differences in percent fat mass among control, C75, or pair-fed mice. Values are means ± SE. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. 

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reduced but not eliminated in the pair-fed animals (Fig. 4B). The C75-treated animals had no identifiable fat accumulation (Fig. 4C) and no histological evidence of liver pathology, such as inflammation or hepatocyte injury. C75 significantly reduced hepatic triglycerides (4.3 ± 0.4 mg/g wet tissue) compared with vehicle control (7.18 ± 1.1 mg/g wet tissue, P = 0.036; Fig. 4D). Compared with DIO mice, lean control mice had significantly reduced triglycerides (3.69 ± 0.28 mg/g wet tissue, P = 0.025). Lean control mice did not develop steatosis and C75 did not significantly alter hepatic triglycerides compared with controls (data not shown).

Hypothalamic orexigenic and anorexigenic peptide expression in C75-treated lean and DIO mice. Our earlier assessment of the effects of C75 on hypothalamic peptide expression was conducted using female lean BALB/c mice. To exclude possible differences between gender or mouse strains, we treated lean C57BL/6J male mice acutely with 20 mg/kg C75, vehicle, or with 23 h of food deprivation to compare their responses to those of BALB/c female mice. Similar to female BALB/c mice, C75 prevented the fasting-induced increased expression of NPY (P < 0.0001) compared with food-deprived mice or ad libitum-fed controls (P = 0.0043; Fig. 5A). Moreover, there was a significant reduction in AGRP expression compared with food-deprived mice (P = 0.0078). POMC and CART mRNA levels were not affected by food deprivation or acute C75 treatment. These data are consistent with our previous findings and indicate that there are no significant strain or gender differences in the hypothalamic neuropeptide responses to acute C75 treatment of lean mice (11).

The effect of C75 on hypothalamic neuropeptide expression was quite different in DIO mice compared with lean mice. In contrast to the results in lean mice, food deprivation did not result in increased levels of hypothalamic NPY or AGRP message in DIO mice (Fig. 5B). Acute C75 treatment increased AGRP expression compared with both vehicle control (P = 0.011) and food-deprived (P = 0.018) animals. C75 also reduced POMC expression compared with vehicle controls (P = 0.05). This pattern of neuropeptide changes of increased orexigenic and decreased anorexigenic peptide expression consistent with a hunger response was not anticipated. This expression pattern obtained 24 h after C75 treatment may indicate a rebound from C75 in acutely treated DIO animals by 24 h after treatment. Interestingly, however, the hypothalamic expression patterns reflected the differences between DIO and lean mouse weight loss and food consumption during the first week of C75 treatment during the 1-mo study. In the 1st wk, the lean mice reduced food intake and lost weight more rapidly compared with DIO mice, which maintained a stable weight (Fig. 1).

We next analyzed hypothalamic peptides by quantitative Northern analysis after 2 wk of treatment. Although the acutely treated lean mice showed dramatic changes in neuropeptide expression, the chronically treated lean animals (Fig. 5, C and E) showed no statistically significant changes. Mice killed 4 h after the last dose of drug (to capture any acute changes that may have occurred) showed a slight increase in CART and POMC expression compared with pair-fed animals, but these changes were not statistically significant (Fig. 5C). Similarly, in mice analyzed 18 h after the last dose of C75 (Fig. 5E), there were no significant differences in neuropeptide expression with chronic treatment. The lack of an anorexigenic pattern of neuropeptide expression after 2 wk mirrors the weight loss seen in the chronic treatment model. Most of the weight loss in the lean mice occurred within the initial 2 wk, during which there was also a substantial reduction in food intake (Fig. 1, C and D). The absence of an anorexigenic neuropeptide profile after 2 wk of C75 treatment may account for the slowed pace of weight loss during the remainder of the experiment.

![Fig. 4. C75 reverses hepatic steatosis. Hematoxylin- and eosin-stained histological sections of liver from control (A), pair-fed (B), and C75-treated (C) mice from the 1-mo treatment experiment. Note intracytoplasmic large and small droplet fat accumulation most prominent in the control animal (arrows). Pair feeding reduced steatosis (arrows), whereas C75-treated animals had no identifiable fat accumulation (400×). D: average hepatic triglyceride measurements from the same animals (n = 5 per treatment group). C75-treated animals had significantly reduced hepatic triglyceride compared with control mice (P = 0.039, unpaired t-test). Individual triglyceride measurements (mg/g wet tissue) from livers depicted in A-C were as follows: control = 6.73, pair fed = 5.35, C75 treated = 2.95. Values are means ± SE. *P < 0.05.](http://ajpendo.physiology.org/doi/10.1210/ajpendo.287-2004-1869)
slow drift upward of food intake at this dose of C75. Interestingly, however, despite the lack of anorexigenic peptide expression, weight did not increase during the last 2 wk. The pattern of neuropeptide expression after 2 wk of C75 treatment in the DIO mice again differed from that obtained in the lean mice. Chronic pair feeding resulted in reduced POMC expression relative to control values. Chronic C75 treatment of DIO mice resulted in a dramatic increase in CART expression compared with vehicle control or pair-fed mice ($P = 0.043$, 1-way ANOVA). POMC expression was reduced in the pair-fed animals compared with control ($P = 0.031$, one-tailed t-test), but no significant change was noted with C75-treated mice. The orexigenic peptides AGRP and NPY showed no significant changes in expression.

We also analyzed hypothalamic peptides after 1 mo of C75 treatment by use of real-time RT-PCR 4 h after the last dose of C75. The C75-treated lean animals showed a profile similar to that of the 2-wk treatment. The C75-treated DIO mice lost the increase in CART expression seen after 2 wk of treatment, indicating a blunting of the anorexigenic response (data not shown). By the end of the study, the DIO mice were no longer losing weight, and their hypothalamic profile was similar to that of the lean mice at 2 wk.

**DISCUSSION**

The pharmacological modulation of FAS and CPT I activities, here accomplished with C75, can reduce food intake and increase peripheral energy expenditure to produce weight loss
over chronic administration. In DIO mice, 1 mo of C75 treatment resulted in sustained, stable weight loss persisting for 2 wk. Interestingly, most of the weight loss occurred during days 10–16 of C75 treatment and corresponded to an anorexigenic hypothalamic peptide profile measured after 14 days of C75 treatment featuring a significant increase in CART expression. In contrast, acute treatment of DIO mice with C75 failed to show a significant anorexigenic response, which was reflected in the slow initial onset of weight loss and reduction of food intake in the chronic model. After 1 mo, when there had been no significant weight loss for 2 wk, there was a loss of the anorexigenic profile. However, control DIO mice continued to increase body mass compared with the C75-treated mice over the course of the experiment.

In lean mice, C75 also produced substantial weight loss, but with a different time course than that seen in the DIO mice. The lean mice lost weight from the outset, along with a dramatic reduction in food intake. Accordingly, the acute 24-h hypothalamic peptide profile showed a significant anorexigenic pattern highlighted by a reduction in NPY and AGRP, as seen in lean BALB/c female mice (6, 11). The differences between our results and those published by another group may reflect dosage or strain differences (8). As most of the weight loss in the lean animals occurred within 14 days, analysis of the expression of hypothalamic neuropeptides after 2 wk and after 1 mo of treatment demonstrated an absence of the anorexigenic response seen in the acutely treated mice. The differences in C75-induced weight loss between DIO and lean mice may be due in part to differences in neuropeptide profiles between DIO and lean mice, which may be important to consider in strategies for weight loss therapies (1, 9, 18, 24, 25).

Although reduction in food intake is an important component of the mechanism of C75-induced weight loss, increased fatty acid oxidation accounts for the significantly increased weight loss in C75-treated mice compared with pair-fed controls (21). In vitro C75 treatment of 3T3-L1 adipocytes, rat hepatocytes, and MCF7 human breast cancer cells increased CPT I activity, fatty acid oxidation, and ATP levels (21). Similar results have been obtained with human adipocytes and myocytes (Thupari JN, unpublished observations). Chronic C75 treatment of both DIO and lean mice also demonstrated the dual nature of C75 action, namely, increased energy expenditure as fatty acid oxidation in the setting of reduced food consumption. C75-treated DIO and lean mice both lost more body mass than pair-fed controls, implying increased energy expenditure. Indirect calorimetry demonstrated increased fatty acid oxidation in both the DIO and lean mice, albeit through different means. In the DIO mice, C75 significantly increased energy expenditure (VO2) compared with pair-fed controls, while maintaining an RER of ~0.7 consistent with fatty acid oxidation. C75 thus increased energy expenditure as fatty acid oxidation in the setting of reduced food consumption, corroborating our initial short-term studies in DIO mice (21). In lean mice, however, C75 did not increase energy production; rather, it increased the duration of fatty acid oxidation as measured by RER while maintaining VO2 levels similar to control. Notwithstanding the different patterns of increased fatty acid oxidation in these animals, C75 treatment caused increased weight loss over pair-fed animals in both DIO and lean mice. Taken together, these data indicate two mechanisms acting in tandem to promote weight loss: a background increase in fatty acid oxidation, with superimposed changes in feeding behavior accompanied by changes in the expression of hypothalamic peptide.

Other studies have demonstrated fundamental differences in hypothalamic feeding peptide responses between lean and DIO rodents (1, 9, 18, 24, 25). For example, a high-fat diet can produce compensatory changes in hypothalamic gene expression in mice resistant to DIO, but not in mice susceptible to DIO (1). Others have shown that POMC induction may attempt to mitigate obesity (25). Consistent with these models, C75 treatment had qualitatively different effects on hypothalamic neuropeptide response in lean and DIO mice. The observation that differences in the hypothalamic response to C75 between lean and DIO mice are qualitative rather than quantitative suggests differential central mechanisms of action of C75 between lean and DIO mice and further supports the idea that the hypothalamic response is altered by changes in diet and/or body weight (1, 9, 18, 24, 25).

Increased VO2 and fatty acid oxidation have now been demonstrated in both acute and chronically treated mice. We have attributed this increase in fatty acid oxidation to a direct effect of C75 on CPT I activity in the peripheral tissues on the basis of in vitro stimulation of CPT I in human and rodent cell lines (21). There remains the possibility that the increased peripheral metabolism is also mediated through the central nervous system via sympathetic afferent outflow. However, we have not been able to detect increased VO2 consumption with intracerebroventricular administration of C75 in lean or DIO mice (Kuhajda FP, unpublished observations). In either case, increased energy production remains a significant peripheral mechanism of action of C75.

Body composition analysis of the DIO and lean mice by MRS indicated that most of the weight loss in the DIO mice was attributable to a loss of adipose mass. Substantial loss of adipose tissue is key for the utility of this approach in obesity management, because a predominant loss of lean mass in obese subjects would be undesirable. In lean mice, however, lean mass accounted for most of the weight loss, limiting the utility of this approach to obese subjects.

In the DIO mice, the reduction in adipose tissue mass was accompanied by reversal of hepatic steatosis without evidence of hepatocellular injury or inflammation. Chemical measurement of liver triglycerides corroborated the histological analysis; C75 significantly reduced liver triglycerides compared with control DIO mice. In human obesity, hepatic steatosis is often accompanied by steatohepatitis, which may progress to cirrhosis and life-threatening liver disease. Resolution of hepatic steatosis without liver injury is an important adjunct to the loss of adipose tissue.

Understanding the mechanism of action of C75 is vital to establishing FAS inhibition and CPT I stimulation as strategies for obesity therapy. We have developed a chronic C75 treatment protocol that enables the investigation of long-term changes in metabolism and hypothalamic neuropeptide expression in both lean and DIO mice. Moreover, because C75 treatment at doses employed in this study do not evoke conditioned taste aversion in mice (6), measurements of both food intake and metabolism in this model can be attributed directly to C75-mediated changes in neuropeptide expression, not to nonspecific effects of sickness behavior. C75 reduced food consumption, increased energy metabolism, and reduced adi-
pose tissue in DIO mice. Moreover, these studies revealed potential fundamental changes in hypothalamic function occurring with changing diet and body habitus that can be utilized for obesity therapies.

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